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In Silico Design of Smart Binders to Anthrax PA

by Michael S. Sellers and Margaret M. Hurley

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In Silico Design of Smart Binders to Anthrax PA

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ABSTRACT

The development of smart peptide binders requires an understanding of the fundamental mechanisms of recognition which has remained an elusive grail of the research community for decades. Recent advances in automated discovery and synthetic library science provide a wealth of information to probe fundamental details of binding and facilitate the development of improved models for a priori prediction of affinity and specificity. Here we present the modeling portion of an iterative experimental/computational study to produce high affinity peptide binders to the Protective Antigen (PA) of *Bacillus anthracis*. The result is a general usage, HPC-oriented, python-based toolkit based upon powerful third-party freeware, which is designed to provide a better understanding of peptide-protein interactions and ultimately predict and measure new smart peptide binder candidates. We present an improved simulation protocol with flexible peptide docking to the Anthrax Protective Antigen, reported within the context of experimental data presented in a companion work.

Keywords: flexible docking, anthrax, binding affinity, peptide binder, *in silico* design

1. INTRODUCTION

The core of a biosensor is a recognition element, carefully chosen to react with a target analyte such as an antigen or contaminant. While biological recognition elements are common, the lure of improved characteristics (increased stability, better affinity, improved specificity) makes synthetic recognition elements such as aptamers and peptides an intriguing alternative. With this in mind, researchers at U.S. Army's *Army Research Laboratory* and *Edgewood Chemical and Biological Center* are endeavoring to design smart peptide binders for enhanced affinity and specificity to the anthrax (*Bacillus anthracis*) protective antigen.

A fundamental understanding of molecular recognition is critical to the design of improved synthetic biosensors. This study utilizes a combined experimental and computational approach to probe the details of the binding. By utilizing an iterative process to successively predict, analyze, and retune the model, we hope to develop an improved computational protocol which correctly reproduces the essential physical elements of the peptide-protein interaction and is capable of reliable *a priori* prediction of peptide binding affinity and specificity.

With this end in mind, the computational side of this study faces a significant challenge. The problem of finding the natural orientation of two complexed moieties is known as "docking" in the modeling community. Over the last two decades, the docking problem has spurred considerable interest and software development, and researchers have worked to overcome the high number of rotational and translation degrees of freedom associated with the binding of two molecular partners. This is compounded by the fact that early docking algorithms were developed based on the classic enzyme-substrate lock-and-key motif. When the aggregation of two larger biological entities is involved, such as a protein-protein or peptide-protein complex, the resulting image is actually more similar to the association of two balls of yarn.

A prodigious number of docking codes is available to the interested user, with varying strengths and weaknesses which are carefully analyzed by the community in open competition such as the CAPRI (Critical Assessment of Prediction of Interactions) competition.[1] It is beyond the scope of this paper to provide a detailed review of docking software, and we restrict ourselves to codes covered within the scope of this project, such as GRAMM-X[2] and PTOOLS[3]. Ultimately the *Rosetta Software Suite* was utilized as the core docking component of our toolkit.[4] The powerful suite is a multi-group supported effort, with numerous capabilities including improved a conformational sampling for both

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sidechain and backbone. This is of interest, as small proteins and short peptide chains that infrequently hold tertiary structure are difficult to dock via current methods. As delineated in several recent works by Baker and Gray, it is vital to the accuracy of the result to include both the flexibility of these peptides and possible changes to the receptor protein.[5] Additionally, the standard description of energetic interactions between target and receptor are not always transferable to such highly flexible partners, and merit further scrutiny.[6]

Accordingly, we have attempted to improve the treatment of flexibility, as well as improved treatment of additional issues such as hydrogen bonding and solvation, through the combination of the multiple computational methods into an HPC-ready, extensible, free, Python-based simulation toolkit . This preliminary work focuses on the initial framework combining the *Rosetta Software Suite* via the PyRosetta script-based interface[7] and the *NAMD* molecular dynamics simulation package developed by the Theoretical and Computational Biophysics Group in the Beckman Institute for Advanced Science and Technology at the University of Illinois at Urbana-Champaign[8]. The current combined computational/experimental effort of designing *smart* peptide binders to the Anthrax protective antigen provides an excellent case study for the development of this toolkit. In the *Methodology* section, we briefly describe the peptides under analysis and the Anthrax protective antigen protein, and provide an outline of the computational protocol, which is dubbed the *XPairIt toolkit*. Our flexibility study and docking results are presented in *Results*, and we identify possible binding locations of the peptide. In *Conclusions*, we discuss our docking results and present a path for future work.

2. METHODOLOGY

The *XPairIt* toolkit also interfaces with the *APBS simulator* for improved electrostatics[9], the *STRIDE* secondary structure prediction tool to assess structural changes during dynamics and on binding[10], and the *PSFGen* structure builder included with VMDand NAMD. A discussion of the *XPairIt* protocol for global and focused docking is described below. A more complete review of the protocol is in preparation.

2.1 Target Analyte-Recognition Element System

The starting structure of the target analyte, the Anthrax protective antigen (PA) is derived from the experimental structure of Petosa et al [12] available in the RCSB PDB (1ACC)[13]. The 1ACC structure is missing residues, and significant work was done to return the protein to its soluble form. We direct the reader to the aforementioned future work for a detailed description of the process. Following this, the PA protein structure is solvated and prepared for docking with a 6 nanosecond(*ns*) molecular dynamics simulation in the NPT ensemble (constant particle number, pressure, and temperature) at 300K, with the CHARMM force-field[14] and TIP3 water. The resulting structure at 6*ns* is then minimized.

The recognition element, a 15-mer peptide developed by experimental collaborators within the context of this work and here referred to as DS23, is constructed within VMD and prepared in a fashion similar to the PA.. Representative structures from this trajectory are saved every 10 ps. The minimized protein structure and ensemble of DS23 structures are then fed into our docking algorithm. This process is depicted in *Figure 1*.

2.2 Flexibility-Enhanced Docking Scheme

Our docking procedure is two-level. At the first-level, a DS structure is randomly chosen from the dynamics trajectory described in section 2.1, and paired with the minimized PA protein. The two structures are then randomly rotated around their respective centers of mass. Next, they are moved along the vector *between* their centers of mass until they are in contact (at ~4.0Å), illustrated in *Figure 1-B*. This is repeated 2000 times to create a set of random starting structures.

Once this first-level set is accumulated, **each** of these structures forms the preliminary structure for 20 individual second-level simulations. Each second-level docking simulation begins by randomly rotating the peptide around its center of mass. This rotation is in addition to the first-level rotation, to ensure sufficient sampling of the peptide-protein surface interaction. Next, *RosettaDock* software's main docking method is used to randomly perturb the peptide structure via small translations and rotations of the entire molecule, and an overall energy is computed after each move. The positions of both DS23 and PA sidechains are also perturbed through sampling of *Rosetta's* rotamer library, a process referred to

as “repacking.”[15] After a complete series of trial perturbations is performed, the resulting structure with the lowest energy is saved. This produces a set of 40,000 second-level structures, providing a somewhat static perspective of the dominant interactions in the complex.

To improve the treatment of flexibility of both partners, and to account for relatively small scale induced fit conformational changes, a 1.5 ps molecular dynamics run at 300K in implicit water is then performed on each second-level docked structure. The Generalized Born Implicit Solvation(GBIS) model[16] as implemented within NAMD[17] is used. After the dynamics is complete, the structure is again minimized with *NAMD*, and the sidechains are “repacked” and re-minimized in *Rosetta*. Data from this entire process (energies, hydrogen bonds, and overall changes in positions) at various stages are recorded and reported in *Results*.

With this flexibility enhanced docking scheme, we generate 40,000 configurations of DS23+PA, which are then ranked and analyzed.

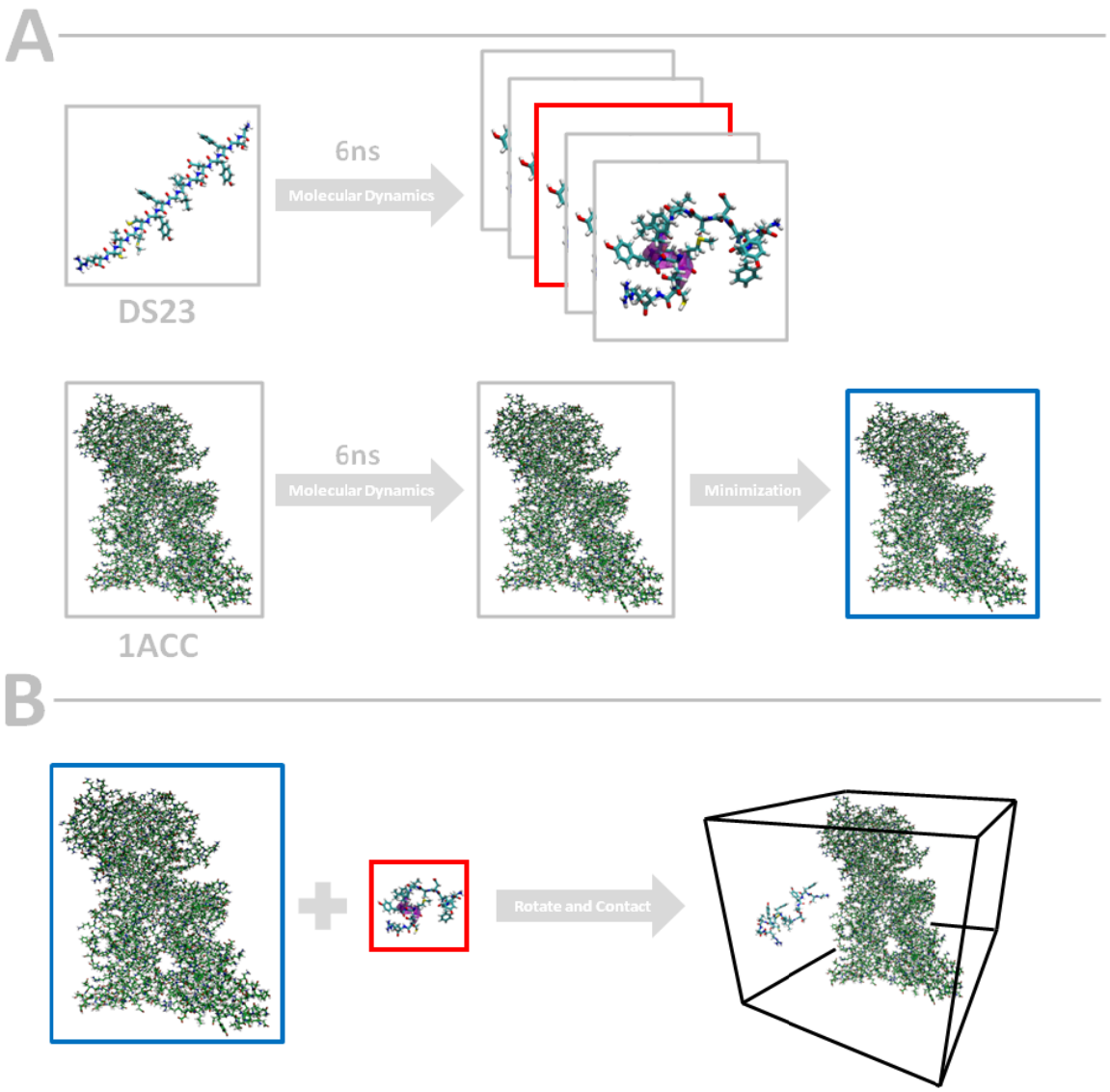

Figure 2.1 (A) Structure preparation procedure. Peptide (DS23) and protein (1ACC→ PA) are simulated separately with molecular dynamics for 6*ns* in TIP3 water with CHARMM. Peptide snapshots are saved every 10*ps*. Protein is subsequently minimized. (B) Initial docking run setup. Structures are rotated around respective centers of mass and placed in contact.

2.3 Docking Analysis

Comparison of energetics and structural clustering are used to parse the resulting 40,000 docked configurations to determine the single structure (or small set of structures) that is most representative of the natural complex of our DS23 peptide and PA protein. In the docking community, computing the energy of a structure for the purpose of comparison to other, similar structures is known as "scoring." Here, each docked structure is scored with *Rosetta's* internal energy function *score12*.[15] However, simple energetic comparison often does not provide a clear indication of the best docked structure—the top 10 structures may have very similar scores, certainly all within the variability associated with thermal fluctuations. Additional information must be used for further refinement.

Accordingly, docked structures are also ranked by their *interface energy*, computed with *Rosetta's score12* function, and the top 25 structures are clustered by peptide binding location over the protein surface. This process is demonstrated within *Results*. The *interface energy* is defined as:

$$E_{Interface} = E_{Total} - E_{Peptide} - E_{\mathrm{Pr}otein} \tag{1}$$

3. RESULTS

Using *Rosetta* docking methods and *NAMD* molecular dynamics within the *XPairIt* toolkit, we generated 40,000 bound configurations of our DS23 peptide and the Anthrax protective antigen (PA). Before the docking runs, the DS23 peptide was simulated using molecular dynamics to generate an ensemble of structures exhibiting the molecule's flexibility. Randomly selected structures from this ensemble were then combined with a full PA protein generated from RCSB's 1ACC structure, and docked according to the scheme outlined in *Methodology*. Results of these docking simulations are analyzed below, and we identify key residues on the DS23 involved in binding, as well as specify a binding location on the PA.

3.1 Analysis of Initial DS23 Dynamics

We briefly provide an analysis of approximately 22*ns* of dynamics (after equilibration) of the DS23 peptide in TIP3 water at 300K. This was performed to assess the native fluctuation of the backbone structure of the peptide, to assess the suitability of a 6ns trajectory for capturing essential backbone motions, and to provide a basis of comparison for structural changes induced upon binding to the PA. A range of properties are used to monitor this behavior, including the *radius of gyration*, *percent helicity*, and a three-residue sequence *helicity parameter* discussed by Speranskiy et al.[18], which provides a measure of a more extended helical character. After equilibration, the *radius of gyration* of this compound fluctuates about an average value of 7.2 Å. This is slightly less than the value of 8.72Å which would be attributed to the same peptide sequence in a perfect helical configuration, and markedly less than the 14.8Å radius of gyration of the beta configuration, or linear value of 16.1Å. It is evident that the structure of the peptide in solvent is quite uniformly compact, although not globular. The end-to-end distance similarly fluctuates regularly about an average of 15.7 Å.

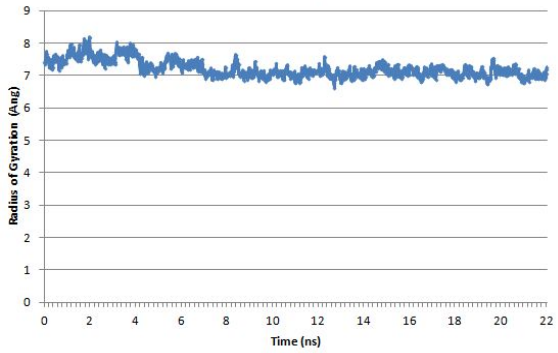

Figure 3.1.1 Dynamic trends of radius of gyration of solvated DS23 vs. simulation time (*ns*).

Within the course of this trajectory, the *percent helicity* (calculated with STRIDE) fluctuates continually and regularly between 0.0 and 66%. The overall average *percent helicity* through the course of the simulation is 34% with a standard deviation of 13%. It is apparent from the stability of the *radius of gyration* value, however, that there is no extension of the protein backbone or uncoiling involved in this process, and this is also evident from visualization of the trajectory. The three-residue sequence parameter of Speranskiy, which we shall abbreviate *H3*, fluctuates regularly between 0 and 5 throughout the same timeframe, with a final average value of 3.04. It is interpreted that the helical nature of the peptide is clustered in an approximate 5-residue sequence, which upon closer examination is seen to exist roughly in the middle of the peptide.

3.2 Ranking of Docked Structures and Structural Similarities

Docked structures of DS23+PA were ranked in two rounds, in conjunction with the two-level docking scheme outlined in *Methodology*. The 20 docked structures generated from each second-level simulation procedure were first ranked by total score (E_{TOTAL} in *Equation 1*). This generated a collection of 2000 top scoring docked structures, clustered over various locations on PA. Next, the structures were ranked by their *interface energy*, as a means to identify highly favorable regions of contact on the PA. Histograms of this second ranking are shown in *Figure 3.2.1*—here, each bin corresponds to a residue on the PA, and a contact distance threshold of 4.0 Å is used to generate the histogram. In *Figure 3.2.1-LEFT*, a contact histogram is shown for the top 25 structures ranked by *interface energy*. PA residues 300-320 show the highest degree of contact. The distribution of peaks throughout the contact map provides evidence that other potential binding locations exist around the PA. By narrowing the analysis to the top 5 structures, shown in *Figure 3.2.1-RIGHT*, a handful of residues on the PA emerge as clear points of contact for the DS23 peptide.

Upon visualization of the top 5 structures, we found that the prospective areas of contact on the PA can be further isolated. For example, the peak near residue 550 in *Figure 3.2.1-RIGHT* also corresponds to peptide contact near residues 300-320 which are in very close proximity on the surface of the PA protein. This leads us to the identification of three spots on the PA that have a high affinity for binding with the DS23 peptide: (1) residues 300-320: *back loop of domain 2* (2) residues 415-425: *domain 2* and (3) residues 680 to 695: *bottom of domain 4*. Of these locations, in 3 of the 5 top docked structures, the DS23 peptide was located near (1), the back loop of domain 2. Additionally, the DS23 in the top scoring docked structure was located near (1).

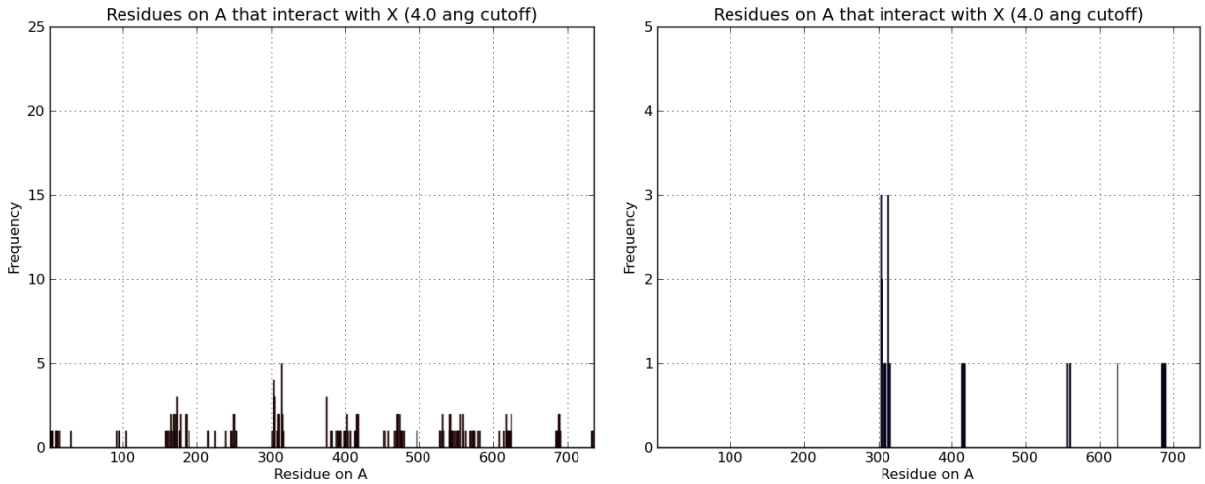

Figure 3.2.1 *LEFT* Contact histogram of top 25 docked structures based on *interface energy*. *RIGHT* Contact histogram of top 5 docked structures based on *interface energy*. Residues on the PA[A] which DS23[X] is in contact with are indicated on the abscissa, frequency on the ordinate.

3.3 Key Residues on DS23 Peptide

We then performed a similar contact analysis of the DS23 moiety for the top 5 docked structures as ranked by *interface energy*. Shown in *Figure 3.3.1* is a contact histogram identifying residues on DS23 that make frequent contact with the

PA in our docked structures. Analysis of the residues with highest contact frequency showed that they are largely located mid-chain, hydrophobic, and planar.

Further study of the single top docked structure showed a similar trend. In *Figure 3.3.2*, the peptide contact histogram of the single top docked structure after an additional short molecular dynamics simulation is shown. Residues 6 and 7 show persistent contact with the PA, as do residues 1, 10, and 12.

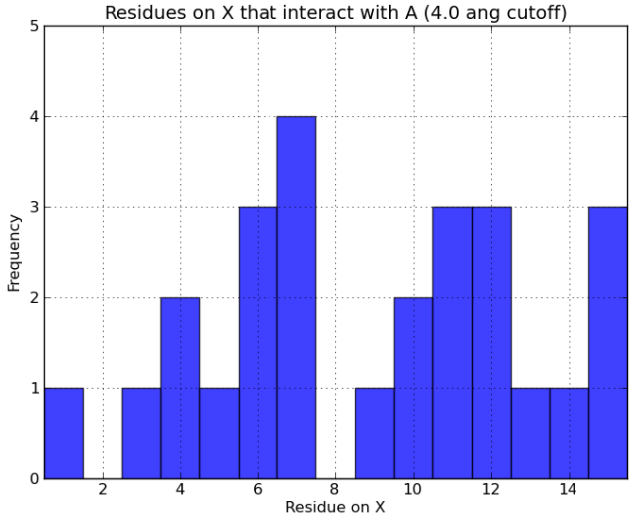

Figure 3.3.1 Contact histogram of top 5 docked structures based on *interface energy*. Residues on DS23 [X] which PA [A] is in contact with are indicated on the abscissa, frequency on the ordinate.

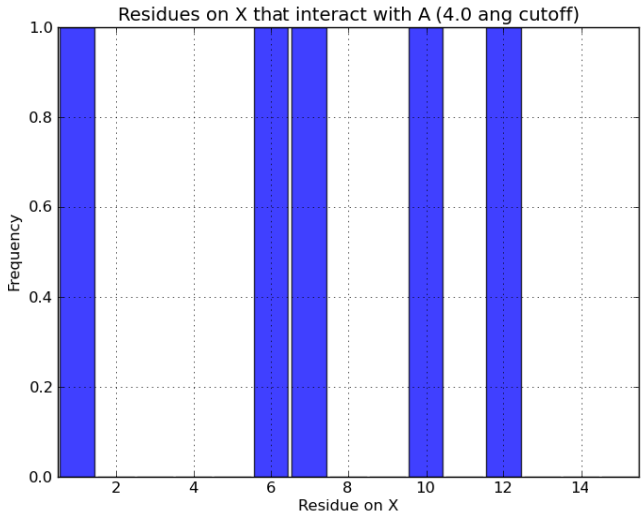

Figure 3.3.2 Contact histogram of top docked structure based on *interface energy*, after a 3*ns* molecular dynamics run. Residues on DS23 [X] which PA [A] is in contact with are indicated on the abscissa, frequency on the ordinate.

3.4 Metrics of Flexibility-Enhanced Docking Scheme

Improvement in the score of overall docking results using a flexibility-enhanced scheme is shown by comparing average score per structure, total number of hydrogen bonds in all analyzed structures, and amount of structure movement of the improved docking protocol, in comparison with the basic *Rosetta* docking method. Shown in *Table 3.4.1* are comparisons of these two stages of our docking scheme. Inclusion of molecular dynamics and minimization within the protocol improves the *interface score* by almost 5 *Rosetta Units* and greatly increases the total number of hydrogen bonds. Considering RMSD/structure (Root Mean Square Displacement per atom, per structure), we do not see a large amount of structural change when using molecular dynamics after *Rosetta* docking. We note that this RMSD does not take into account any large scale fluctuations from the initial dynamics trajectory sampling

Table 3.4.1. Toolkit (T) vs. Standard Docking (D) metrics for top 50 structures.

	Average score/structure (*Rosetta Units*)	**Total Hydrogen Bonds**	**RMSD/structure from (D) to (T) (*Å*)**
Interface Score	-8.07(T) vs. -3.30 (D)	53(T) vs. 2(D)	0.69

4. CONCLUSIONS

The XPairIt protocol was developed to improve docking of flexible biological partners such as peptides and proteins. The premier case study for the software is designing a peptide binder for the Anthrax protective antigen. Preliminary docking results are complete and we continue to collaborate with ongoing experimental work through an iterative approach, allowing for development and refinement of our software toolkit.

Structure and function of several patches on the PA offer an explanation of these preliminary docking results. Our top scoring and most frequent docked structure was that of the DS23 in contact with the PA's domain 2 loop. This highly flexible chain on the PA plays a large role in membrane insertion, and contains several hydrophobic, aromatic residues—specifically, two PHEs that are exposed to the solvent.[12] This loop presents a large surface area for the binding of several hydrophobic residues of the DS23 peptide, and docked structures here exhibit the best *interface energy*—a barrier for desorption of the peptide. This matching of hydrophobic patches is illustrated in Figure 4.1.1 below. Additional preferred locations for DS23 binding appeared in an alternative section of PA's domain 2 and at the base of domain 4. It is noteworthy that these locations also display an appreciable hydrophobic surface. Interestingly, the putative domain 4 binding location, located completely *a priori* from this simulation, is in fact an active binding site to the M18 antibody and CMG2 cell receptor protein.[19, 20] It is encouraging to see the *Rosetta* scoring function and flexibility introduced with *NAMD* drive the DS23 peptide toward these active sites, and highly auspicious that binding features introduced within the dynamics portion of the protocol (such as additional hydrogen bonding) do not disappear under docking repacking and reminimization. However, as all putative binding sites are patently hydrophobic, we question whether the subtleties leading to prediction of specific binding are clouded by the energy expressions used. Work probing this question is ongoing.

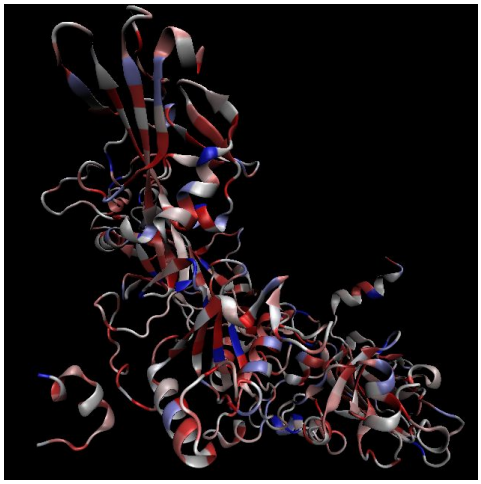

Figure 4.1.1 Top docked structure binding to the domain 2 loop. The DS23 peptide is clearly visible in the lower lefthand side. Coloring is by Eisenberg hydrophobicity scale from red (*hydrophobic*) to blue (*hydrophilic*).

The basic metrics provided demonstrate a marked improvement in our ability to generate *a priori* predictive docked structures. Larger magnitude (more negative) *interface energies* and a greater number of hydrogen bonds indicate the effectiveness of the protocol. The importance and frequency of hydrogen bonding in bound configurations of peptides is illustrated in a recent survey of peptide-protein complexes.[21] Displacement values show, however that this is not a magic bullet. Full resolution molecular dynamics provided only less than 1.0Å RMSD. Here, we acknowledge the aid in PA structural relaxation leading to improved peptide contact, but consider that large-scale structural moves may be better represented by other simulation methodology.

Our work on the DS23-PA system continues, with experimental validation of proposed peptide docking locations and testing mutations of the peptide for binding improvement. From the software perspective, the *XPairIt* toolkit is currently being extended to support a coarse-grained atomistic potential, to incorporate a more rigorous calculation of system electrostatics, and to include capability for quantum mechanics.

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